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PREPARATION AND METHOD OF USING TOXIN-ANTITOXIN MIXTURES FOR ACTIVE IMMUNIZATION AGAINST DIPHTHERIA *

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Inquiries are made frequently for a detailed account of the method of preparing the mixtures of toxin-antitoxin, which have been used by us in the active immunization against diphtheria. The following communication is intended to answer some of the questions.

In our earlier work we began with mixtures of toxin-antitoxin, which were slightly overneutralized. Later, neutralized mixtures were used and finally slightly toxic mixtures were found to give the most satisfactory results.

The substances used for preparing the toxin-antitoxin consist of a well ripened and carefully standardized toxin and a preparation of antitoxin the strength of which has been closely determined.

The Toxin.—It is of advantage to have on hand a supply of from 50-100 liters of diphtheria toxin which has been passed through a Berkefeld filter and allowed to ripen in the ice-box for 6-12 months. The toxin is conveniently stored in the ice-box in liter bottles. During the first 3 months of ripening, a considerable proportion of the toxin is changed into toxoids. The toxin shows less change in its potency during the later months, and finally reaches a point where it is quite stable. By using the toxin at this time for the preparation of the toxin-antitoxin mixture, further changes of any considerable extent in the strength of the mixture will be avoided. The mixture will remain stable and will show unimpaired immunizing qualities for at least 6 months.

It is important to use a strong toxin with an L+ dose of at least 0.4 c.c. Each cubic centimeter of such toxin will represent 2.5 L+ doses. If the toxin is stronger, the number of L+ doses per cubic centimeter will be larger and the immunizing value of the mixture greater. It is difficult, however, to obtain a toxin stronger than one with an L + dose of less than 0.2 c.c. A mixture prepared with a toxin of such strength (L + = 0.2 c.c.) would contain about 5 neutralized doses of toxin and represent a toxin-antitoxin preparation of maximum efficiency. It is, of course, possible to give a larger dose than 1 c.c. of a toxin-antitoxin mixture prepared from a weaker toxin, and thus administer a similar number of neutralized L + doses. By increasing the volume of the dose, however, we also increase the amount of foreign protein injected, especially in the case of the diphtheria bacillus. This is the particular agent involved in producing, in susceptible persons, the more severe types of local and constitutional reactions. In using a strong, ripened toxin, with an L + dose of 0.4 to 0.2 c.c., representing 2.5 to 5 L + doses of toxin in each cubic centimeter, we can prepare a suitable mixture of

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toxin-antitoxin. Within these limits the mixture will be efficient for purposes of active immunization.

The Antitoxin.—It is advisable to set aside 100-200 c.c. of a concentrated preparation of antitoxin, the strength of which has been closely determined. With a sufficient supply of standardized toxin and antitoxin on hand, additional quantities of toxin-antitoxin may be readily and accurately prepared.

The Toxin-Antitoxin.—After determining the amount of antitoxin to be added to the liter of toxin, some of the concentrated antitoxin is diluted 1:10 with sterile physiologic sodium chlorid solution to facilitate the exact measurement of the necessary number of units of antitoxin. To prepare a slightly toxic mixture, in which the desired proportion of toxin is 85% of an L+ dose to each unit of antitoxin, the amount of antitoxin necessary to be added to 1 L+ dose of toxin will be 1.17 units. For example: a toxin with an L+ dose of 0.4 c.c. contains 2.5 L + doses per cubic centimeter and will require 2.5 × 1.17 or 2.9 units of antitoxin to each cubic centimeter, and 2900 units to the liter of toxin. Where a stronger toxin is used, proportionately more antitoxin will have to be added.

After the mixture of toxin and antitoxin is thoroughly shaken, it is allowed to stand for 2 hours before sterility and potency tests are made. The toxicity is carefully determined in 2 guinea-pigs, one of the animals receiving subcutaneously 1 c.c., the other one 5 c.c. of the toxin-antitoxin. The animal receiving the smaller dose should show a slight induration; the other one injected with the larger amount should show a moderate or marked local induration and late paralysis, but should not die acutely of diphtheria poisoning. If the animal dies before the 5th day, it indicates an excess of free toxin in the mixture, which has to be carefully neutralized. It is evident, therefore, that to facilitate the quick preparation of additional amounts of toxin-antitoxin of uniform strength, it is necessary to have on hand a sufficiently large supply of the same preparations of toxin and of antitoxin.

Bottling and Labelling.—If the preparation is sterile and of proper strength, it is then put up in 10 c.c. bottles, and kept cold in the ice-box. We have found it of advantage, for purposes of identification, to give each lot of toxin an alphabetical letter, to which is added a number to designate each liter of the toxin-antitoxin mixture.

Relative Value of Various Mixtures of Toxin-Antitoxin.—A toxin which is excessively overneutralized and contains to each L + dose from 10 to 100 units or more of antitoxin, will produce an active immunity in only a small proportion of nonimmunes. We have found that children, who were exposed to diphtheria and were given for purposes of immediate protection 1000 units of antitoxin with the 1st or the 2nd dose of toxin-antitoxin, did not respond as well to active immunization as those who received the toxin-antitoxin alone. This is due to an overneutralization of the toxin, which greatly impairs its immunizing value. The diminution in the antitoxin response after the injection of an overneutralized toxin has also been shown in the case of horses. These animals show, as a rule a much better antitoxin production after the injection of a mixture of diphtheria toxin-antitoxin, which contains 1.5 units of antitoxin to each L + dose of toxin, than after the injection of a mixture containing 6 units of antitoxin to each L+ of toxin. On the other hand, a mixture of toxin-antitoxin which is underneutralized or toxic is dangerous to use on account of the excess of free toxin.

Mixtures which are either slightly overneutralized, neutral, or slightly toxic are safe and will give rise to an active immunity. Of the three, the slightly toxic mixture will show the better antitoxin response, without producing any more local or constitutional symptoms than the neutral or overneutralized mixtures.

Table I shows the amounts of toxin and of antitoxin, and the percentage in each mixture of the L+ dose of toxin to one unit of antitoxin.

Dose and Method of Administration.—The mixture, as above described, is used undiluted. The dose is 1 c.c. injected subcutaneously in the arm at the insertion of the deltoid. The dose is repeated at weekly intervals until 3 injections have been given. For children under 1 year, the dose is 0.5 c.c. In the younger children, the local and constitutional symptoms following the injections of toxin-antitoxin are much less marked than in older children and adults. The difference is due to a greater susceptibility of older persons to the autolyzed protein of the diphtheria bacillus, which is present in the mixture of toxin-antitoxin.

Determination of Immunity Response.—The development of an active immunity is determined with the Schick test at the end of 3 months. The Schick retest is delayed for this period of time, so as to include all persons who will become actively immune. The results of some previous work carried out on numerous groups of persons have shown that the development of antitoxin in many persons is often a slow process, requiring 8-10 weeks before a sufficient amount is produced to inhibit the Schick test. The number of successfully immunized persons who finally show a negative Schick retest after 3 injections of toxin-antitoxin is 90-95%. After only 1 or 2 injections of toxin-antitoxin, a smaller number show the development of an active immunity: after 1 injection, about 60%; after 2 injections, about 80%. By reinjecting those who still give a positive Schick test, at the end of 3 months, with 2 or 3 more doses of toxin-antitoxin, an active immunity may be developed in almost all susceptible persons. In using the Schick test as an index of immunity, it is important not to mistake a pseudoreaction for a persistent positive Schick test.

TABLE 1

MIXTURES OF DIPHTHERIA TOXIN-ANTITOXIN

Type Mixture	Amount of Toxin	Amount of Antitoxin in Units	Percent. L+ to 1 Unit of Antitoxin
1. Slightly toxic	1 L +	1.125—1.25	80-90
2. Neutral	1 L +	1.25 —1.5	65-80
3. Slightly overneutralized	1 L +	1.5 —2.0	50-65

The indications for active immunization with toxin-antitoxin may be divided into 2 groups: (a) as a general prophylactic measure, and (b) to control outbreaks of diphtheria.

The most suitable age period for prophylactic immunization is between 6 and 18 months. At this time of life nearly all children are susceptible to diphtheria. While only 15% give a positive Schick test at birth, almost all the remaining children lose their maternal immunity after the 6th to 9th month of life and then show a positive Schick reaction. It is, therefore, a safe and convenient rule to follow to immunize with toxin-antitoxin all children below 18 months of age irrespective of the Schick test they may show at the time of immunization.

There are several advantages in this procedure. First, by omitting the Schick test in this group of children, the immunization will be greatly simplified and more readily applied by the large majority of general practitioners. Secondly, the local reaction after the toxin-antitoxin in young children is very slight or absent. Third, the morbidity and mortality from diphtheria is greatest between 1 and 4 years of age; therefore an early immunization on a large scale of young children is of great importance in controlling the disease. Fourth, the immunity which develops after injections of toxin-antitoxin is an active immunity, that persists for several years and possibly for life.

After the age of 18 months it is of advantage to apply the Schick test first, so as to exclude from immunization those children who show a negative reaction and are probably permanently immune. Young children can be reached in the homes, in infant asylums, in the milk stations, and in day nurseries. The children of the next age period are included in the preschool and school groups. These children may be reached in the public schools, in orphan asylums, and in the various other institutions. Among adults, those who come in contact with diphtheria and are constantly exposed and in danger should also be tested and immunized with toxin-antitoxin, if found to give a positive Schick reaction. Included in this group are especially physicians, nurses, and hospital attendants in contagious-disease hospitals.

The immunity developing from toxin-antitoxin is slow in appearing, and is, therefore, not a reliable safeguard in hospital wards where children are crowded together, and suffering from various contagious diseases. But in institutions where small outbreaks of diphtheria have occurred, or where diphtheria is more or less constantly present and clinical cases and bacillus carriers steadily appear, the use of antitoxin alone has often been insufficient to stamp out the disease, and the combined application of the Schick test and active immunization with toxin-antitoxin has given most successful and encouraging results.